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Archeo-Cell Biology: Carbon Dating Is Not Just for Pots and Dinosaurs

Defining the life span of specific human cell populations is limited by our inability to mark the exact time when cells are born in a way that can be detected over many years. In this issue of *Cell,* Spalding et al. (2005) describe a clever strategy for retrospectively birth dating human cells in vivo, based on their incorporation of ^{14}C during a peak in atmospheric levels of this isotope resulting from above-ground nuclear arms testing in the 1950s.

An unresolved issue of interest to many biologists is how to measure the life span and rate of turnover of particular cell types in the human body (and in other long-lived animals). How fast do different cell types age, and how often are cells replaced in different organ systems in vivo? Answers to these questions require understanding of the rates of decay and regenerative potential of different tissues in the body. In practical terms, the intrinsic life span of particular cell types relates directly to disease, degeneration, aging, and the potential for cellular regeneration. These factors, in turn, affect the development of experimental approaches for therapeutic replacement of diseased cells.

Most of what we know about the life cycle and regenerative potential of different mammalian cell types comes from studies in nonhuman species (particularly rodents). Such studies rely on either molecular markers of cell division to define the mitotic activity of particular cell types, or methods in which newborn cells are labeled by incorporation of modified nucleotides into replicating DNA during S phase of the cell cycle (Nowakowski and Hayes, 2000). These methods have clear strengths when used in short-lived laboratory animals but severe limitations when investigating the life cycle of human cells in vivo.

Although markers of cell division are useful for identifying the proliferation state of individual cells or populations of cells, they are also limiting because they provide a static picture at a single time point. In addition, cell division markers do not provide insight into the past history of cells and thus cannot be used to determine when the cells were born. In contrast, administration of radioactive or modified nucleotides—historically, ^{3}H-thymidine, but now more commonly bromodeoxyuridine (BrdU) or similar halogenated uridines—at the time of DNA synthesis during cell division labels newborn cells and allows them to be detected indefinitely. These approaches have proved reliable for labeling newborn cells in rodents, birds, and nonhuman primates, even in tissues in which cell turnover is quite limited, such as the neuronal populations of the central nervous system (CNS). Nonetheless, the toxicity of these compounds all but eliminates their use in humans (Eriksson et al., 1998). Data collected in rodents can only partly be translated to humans because cell life span and rates of cell aging are quite different between the two species.

Intriguing questions persist about cell life span and turnover. Do some cell populations, such as most CNS neurons, survive the roughly century-long duration of human life without being replaced? Do cell types that are thought to be immutable undergo replacement over years or decades? Does disease alter the regenerative capabilities of cell types that normally do not regenerate?

In this issue of *Cell*, Spalding and coworkers (Spalding et al., 2005) describe a broadly applicable strategy for determining the birth dates of human cells that starts to provide answers to some of these questions. In a clever and elegant manner, the authors take advantage of a peak in the carbon isotope,^{14}C, that accumulated in the atmosphere between the mid 1950s and early 1960s as a byproduct of above-ground testing of nuclear weapons. Such testing was banned by treaty in 1963, and ^{14}C levels in the atmosphere dramatically declined in the following decades to previous low background levels. The use of ^{14}C to date archeological material and ancient artifacts is familiar, based on its natural accumulation in the biosphere and its slow rate of decay (the half-life of ^{14}C is >5700 years).

Using postmortem tissue samples from Swedes born before or after the nuclear bomb tests, Spalding and colleagues analyzed integration of ^{14}C into genomic DNA during each cell's last division and calculated the birth dates of different cell types collected from human intestine, skeletal muscle, cerebellum, and cerebral cortex. They demonstrate that this approach is accurate to within a few years and is applicable to postmortem specimens collected more than 50 years after DNA replication occurred in some cell populations. The idea is simple. Given that atmospheric ^{14}C was extremely low before the era of above-ground nuclear testing and sharply decreased after 1963 (the end of nuclear testing), and given the virtual absence of decay of ^{14}C incorporated into genomic DNA over this short 50 year period, the amount of ^{14}C in postmortem tissues directly reflects the amount of ^{14}C in the atmosphere when the cells underwent their last division.

The authors first needed to determine the amount of atmospheric ^{14}C from the mid-1950s to the present, which they did by measuring the amount of ^{14}C in individual annual growth rings of pine trees in Sweden. This provided a temporal scale with which to establish atmospheric ^{14}C levels over the last 60 years. A peak of

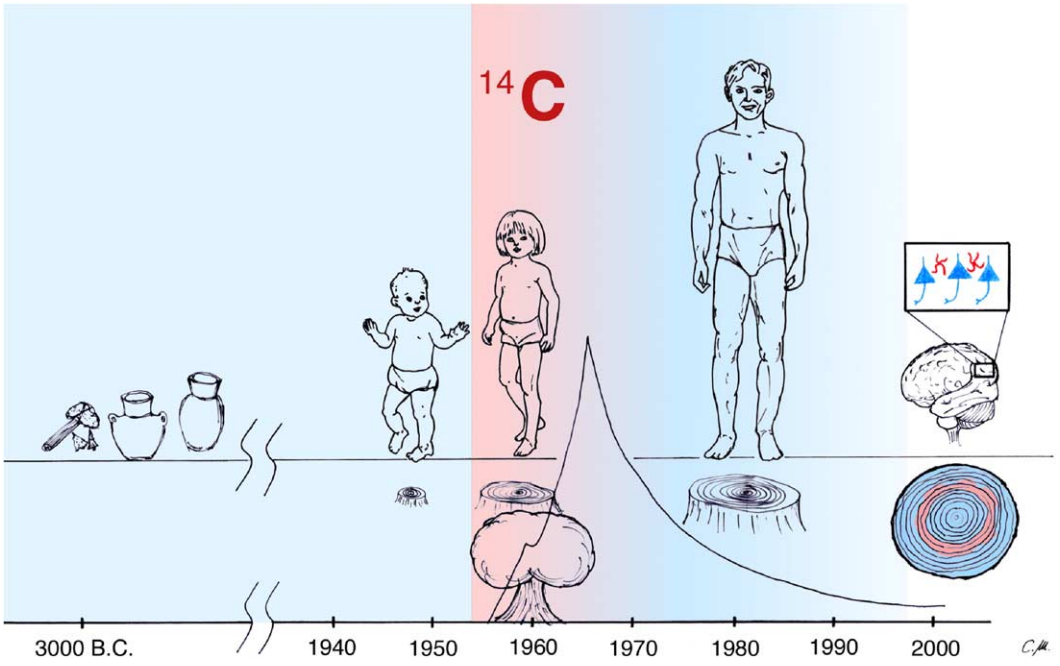

Figure 1. Retrospective Birth Dating of Human Neurons

The amount of ^{14}C in neurons of the human cerebral cortex (blue) corresponds to the amount of ^{14}C found in the atmosphere at the time of birth of the individual. This shows that there is minimal turnover of neurons during postnatal and adult life. Nonneuronal cells are younger, as shown by higher levels of ^{14}C (red). The amount of ^{14}C in the atmosphere corresponds to the amount found deposited in the rings of pine trees in Sweden, the geographic area studied (Spalding et al., 2005). Illustration by Claudio Mare, Somerville, Massachusetts.

^{14}C was recorded for the years of above-ground nuclear testing (see Figure 1). Because the total amount of ^{14}C in organisms mimics the amount of ^{14}C in the atmosphere at any particular time, the birth date of a group of cells can be determined by finding the year during which atmospheric ^{14}C content matches the concentration of ^{14}C found in the cells (see Figure 1). The authors use this strategy to directly address a controversial question in neuroscience: Are neurons of the human cerebral cortex replaced over the course of a human's life span, or do the same neurons persist for 100 years or more?

The idea that spontaneous turnover of neurons in the adult CNS is maintained after embryonic development is completed is a controversial one. Seminal discoveries in rodents clearly show that neurogenesis (the birth of new neurons) occurs constitutively in two regions of the adult mammalian brain: the olfactory bulb (from precursors in the subventricular zone near the lateral ventricles) (Lois and Alvarez-Buylla, 1993; Luskin, 1993) and the dentate gyrus of the hippocampus (Altman and Das, 1965; Eriksson et al., 1998; Kuhn et al., 1996). Although constitutive neurogenesis in these two regions is generally accepted, it is still debatable whether other regions of the adult CNS of mammals exhibit such neuronal birth.

The cerebral cortex is the center of language, cognition, and the high-level associative functions that distinguish us as humans. It is the most complex region of the brain and is characterized by marked cellular heterogeneity and myriad neuronal connections. Historically, the cerebral cortex has been thought of as immutable and hard wired, but this view has been challenged by the discovery that synapses change dramatically over time and that neural precursors (often termed "stem cells") exist in the subventricular zone underlying the cerebral cortex. These stem cells can be isolated from the adult cerebral cortex and give rise to new neurons in vitro (Palmer et al., 1999). Indeed, small numbers of new cortical projection neurons can be induced in adult mice even though there is no constitutive neurogenesis in the adult mouse cerebral cortex (Chen et al., 2004; Magavi et al., 2000). The notion of de novo neurogenesis under normal conditions in the adult cerebral cortex is controversial. Several studies suggesting a low birthrate for new adult neurons (Gould et al., 1999; Kaplan, 1981) have been challenged by more recent findings in rodents and nonhuman primates that no neurons are generated after birth in the mammalian cerebral cortex (Kornack and Rakic, 2001; Magavi et al., 2000).

From their postmortem analysis of ^{14}C in tissues from the human occipital cortex, Spalding and coworkers (Spalding et al., 2005) provide convincing evidence that, under normal conditions, essentially all neurons of the human cerebral cortex are born during embryonic or early postnatal development and no neuronal birth occurs in adult life (at least in the specific cortical areas they examined). Their conclusion is supported by their analysis of neuronal nuclei extracted from the occipital cortex of individuals born before the nuclear testing era. Their analysis revealed extremely low ^{14}C levels in

those nuclei typical of the period before nuclear testing began; cortical neurons from people born later have ^{14}C levels that match those in the atmosphere at the time of their birth. This reinforces the longstanding view that the life span of neurons in the human cerebral cortex can be a century or more.

The Spalding et al. (2005) study enables a more direct understanding of cell turnover, aging, and life span throughout the human body and those of other long-lived animals. This, in turn, informs our understanding of human developmental and regenerative biology. Such knowledge will be instrumental in developing cellular replacement therapies to treat damaged and diseased tissues.

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